Industrial Crops & Products 124 (2018) 459–465

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

How extraction method affects yield, fatty acids composition and bioactive properties of cardoon seed oil?

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ARTICLE INFO

Keywords:
Cynara cardunculus L.
Cardoon
Oil extraction
Mechanical pressing
Screw pressing
Hepatotoxicity

ABSTRACT

Cardoon (*Cynara cardunculus* L.; Asteraceae) is a perennial species with several uses, especially in the industry of energy production, while it is classified as a non-conventional energy crop within the European Union (EU). The aim of the present study was to evaluate chemical composition and antioxidant properties of cardoon seed oils extracted with two mechanical pressing methods, while at the same time it further determined composition and antioxidant properties of the obtained seedcakes. Oil extraction yield did not differ between the tested methods and growing years, indicating that both heat and cold extraction are efficient methods for oil production (approx. 75% extraction yield). Oils obtained from heat extraction method had better antioxidant properties than cold pressed oils, while significant variation between the growing years was also observed. Seedcakes of heat extraction method had the highest total phenols (405 mg Gallic acid equivalents (GAE)/g extract) and the highest antioxidant properties for all the tested assays (with the exception of reducing power assay). Moreover, none of the studied materials (seeds, seed oils, and seedcakes) showed toxicity effects against PLP2 non-tumor cells (GI_{50} > 400 μg/mL). The main fatty acids were linoleic and oleic acids which were detected in similar amounts in oils and seedcakes, while significant variation was observed between the tested methods and the growing years. The results of the present study signified the importance of cardoon as an alternative field crop under the Mediterranean climate conditions. In addition, seed oil production byproducts (e.g. seedcakes) are a promising material due to its bioactivities and its fat content and fatty acid composition, that could find alternative uses in the pharmaceutical and cosmetics industry.

1. Introduction

Cardoon (*Cynara cardunculus* L.; Asteraceae) is a perennial species native to the Mediterranean basin, which consists of globe artichoke (*C. cardunculus* var. *scolymus* (L.) Fiori), as well as leafy cardoon; the latter is further divided into two cultivar groups, namely cultivated cardoon (*C. cardunculus* var. *altilis* DC) and wild cardoon (*C. cardunculus* var. *sylvestris* (Lamk) Fiori) (Pagnotta et al., 2017; Raccuia et al., 2011). Cultivated and wild cardoon have been traditionally used for its edible leaf stems (Renna et al., 2018), while in many regions of the Mediterranean plant immature inflorescences are also consumed in various gourmet dishes (Christaki et al., 2012; Fernández et al., 2006), or in the cheese-making industry (Almeida and Simões, 2018). However, during the last decades there is a great interest for industrial applications of cultivated cardoon, focusing on energy and biofuel production, with several studies confirming the great potential of the species for such purposes (Angelini et al., 2009; Grammelis et al., 2008; Vasilakoglou and Dhima, 2014). According to Ferreira-Dias et al. (2018), cardoon seed oil is rich in triacylglycerols, sterols (especially β-sitosterol and Δ^7-stigmastenol), as well as in tocopherols (α- and δ-tocopherols), while they suggested that chemical fingertip of oils may be used for the identification of growing site. Moreover, Curt et al. (2002) carried out a multi-year and multi-location experiment in order to evaluate seed oil production, and reported a great variation in oil yields between experimental locations (20.0–31.6%) and the tested populations (22.0–28.8%), which highlights the pivotal importance of both genetic material selection and climate conditions for the achievement of higher oil yields. In the study of Foti et al. (1999), a significant variation

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https://doi.org/10.1016/j.indcrop.2018.08.027
Received 10 June 2018; Received in revised form 8 August 2018; Accepted 9 August 2018

between two growing years was observed in seed oil content of a wild cardoon landrace, whereas for two cultivated cardoon genotypes the variation was less profound. Moreover, Maccarone et al. (1999) suggested that genotype may have a significant effect on fatty acids composition of seed oils, while they also reported a variation between two growing years.

However, apart from pre-harvest factors seed oil yield could be also affected by the extraction method, where according to recent studies pretreatment of seeds with microwaves prior to cold extraction has been reported to increase oil yield in purslane (Delfan-Hosseini et al., 2017) and rapeseed seeds (Yang et al., 2013). Although solvent extraction is the most efficient method for seed oil extraction, mechanical pressing of seeds is most commonly used in industrial scale oil production due to several drawbacks of solvents extraction technique, including high cost of consumables, environmental burden, laborious processing steps and high production costs (Azadmard-Damirchi et al., 2010). Depending on the solvent used in each extraction method, chemical composition of the obtained oils may differ significantly in terms of phenolic compounds, sterols and fatty acids content (Kozłowska et al., 2016), while differences in chemical composition of seed oils have been also observed between cold and heat-pressed oils attributed to temperature differences during seed processing and oil extraction (Delfan-Hosseini et al., 2017; Siger et al., 2017).

According to Petropoulos et al. (2017) and Petropoulos et al. (2018), apart from industrial use for energy production purposes, cardoon by-products could be used as bioactive compounds sources, thus increasing the added value of the crop. Oilcakes or seedcakes could also consist a valuable byproduct, since according to Bochkarev et al. (2016) seed oil and fat production byproducts of various plants such as pumpkin, flax, milk thistle, and soy present technological and nutritional properties that may find several alternative uses in the food industry. Moreover, Genovese et al. (2016) have highlighted the potential of using cardoon seedcakes as animal fodder which could further increase the overall added value of the crop.

Considering the great variation in cardoon grain yield reported in the literature (0.6–4.3 t ha^{-1}; (Curt et al., 2002; Foti et al., 1999; Gominho et al., 2011), the perennial nature of the species and oil extraction yield for mechanical pressing methods (up to 80% of total oil), the amount of byproducts in the oil production industry raises concerns regarding its management in an environmental friendly manner. Therefore, the aim of the present study was to evaluate oil yield of seeds from cardoon plants grown in the central Greece, with two extraction methods (heat and cold extraction) and in comparison with oil yield obtained with Soxhlet extraction. In addition, fatty acids composition of raw seeds, seed oils and seedcakes in relation to the extraction method and growing year was examined, while antioxidant activity and cytotoxicity were also determined in order to evaluate the potential of increasing the added value of the crop by using the seed oils and byproducts of oil production (seedcakes) as alternative raw materials for the pharmaceutical and cosmetics industry.

2. Materials and methods

2.1. Plant material

A field experiment was carried out at the experimental farm of the University of Thessaly in Velestino (22.756E, 39.396N), Greece during 2014–2015. Seeds of cultivated cardoon [*Cynara cardunculus* L. var. *altilis* DC] cv. Bianco Avorio (Fratelli Ingegnoli Spa, Milano, Italy) were collected from fully mature plants grown from seeds, starting four years after crop establishment (2014). Seeds were collected at the second fortnight of June for both the experimental years (2014–2015), as previously described by Petropoulos et al. (2018). Briefly, 50 cardoon plants with uniform growth were selected and one mature head from each plant (the central head of each compound of heads) was collected at principal growth stage 8 (code stage 89; Archontoulis et al. (2013)) and as soon as the heads were dry and senesced and the seeds fully ripened (Petropoulos et al., 2018). After the harvest, seeds were separated from the heads and batch samples were prepared for oil extraction with different methods, as described below (Section 2.2). Moisture content of seeds was recorded by putting ground whole seed samples in a forced-air oven at 72 °C until constant weight. Moreover, batch samples of whole seeds were ground with an electric ball mill (PX-MFC 90 D, Kinematica AG, Switzerland), freeze-dried and stored in deep freezing conditions (−80 °C) for antioxidant activity and cytotoxicity assays.

2.2. Seed oil extraction methods

For seed oil extraction, two different mechanical methods were implemented including one heat and one cold extraction method. Heat extraction was carried out with seeds collected at two consecutive years (2014–2015), whereas cold extraction was applied only for seeds collected at the last experimental period (2015). More specifically, heat extraction was carried out with the use of a small type screw oil press TäbyPressen Type 40 (Skeppsta Maskin AB, Örebro, Sweden). Oil temperatures during heat extraction ranged between 53–55 °C. Nozzle diameter was 6 mm, while seeds were fed to the screw press at a seed feeding rate of 5 kg/h and a rotational speed of 78 rpm. Cold extraction was carried out by Amygdalea S.A. (Volos, Greece) with the use of a Komet DD 85 G twin screw vegetable oil expreller (IBG Monforts Oekotec GmbH & Co.KG; Mönchengladbach, Germany). Oil temperatures during cold extraction 2 ranged between 40–44 °C. Nozzle diameter was 6 mm, while seeds were fed to the screw presses at a seed feeding rate of 8 kg/h and a rotational speed of 65 rpm. Oil extraction was carried out in triplicate for each tested method. For both methods, samples of oil were collected after 15 min of operation in order to allow the presses to achieve steady operation. After extraction, all seeds oils were centrifuged twice at 3500 × *g* for 10 min. After each centrifugation, the supernatants were collected in a new dark vial and stored at room temperature and dark conditions until further analysis.

Oil yield for each extraction method was estimated as the percentage of extracted oil (g of oil) over the total amount of pressed seeds. For comparison purposes, total seed oil content was estimated with a Soxhlet apparatus. The samples (10.0 g) were extracted with 200 mL of petroleum ether by refluxing in a Soxhlet apparatus, during 8 h (~32 cycles, using a Soxhlet extractor with the capacity of 250 mL) to assess oil recovery. Afterwards, the solvent was removed under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland), in order to obtain the oil content.

Seed cakes after oil extraction were also collected, freeze-dried (FeeeZone 4.5, Labconco, Kansas City, MO, USA), put in air sealed bags and stored at deep freezing conditions (−80 °C) until further analysis. Moisture content of seedcakes was also recorded by putting ground seed samples in a forced-air oven at 72 °C until constant weight

2.3. Fatty acids composition analyses

Fatty acids of seeds, seed cakes and seed oils were analyzed with a DANI 1000 gas chromatographer (GC) coupled to a flame ionization detector (FID) after a transesterification procedure described by Heleno et al. (2009) and results were recorded and processed using Clarity 4.0.1.7 Software (DataApex, Podohradska, Czech Republic).

2.4. Antioxidant activity assays

Extracts from freeze-dried samples of seeds and seed cakes were prepared by stirring the dry sample (1 g) and 30 mL of methanol/water (80:20 v/v, at 25 °C at 150 rpm) for 1 h and afterwards filtered using Whatman paper No. 4. The residue was then extracted with an additional portion of (30 mL) methanol/water and the combined extracts were evaporated under reduced pressure, until complete removal of